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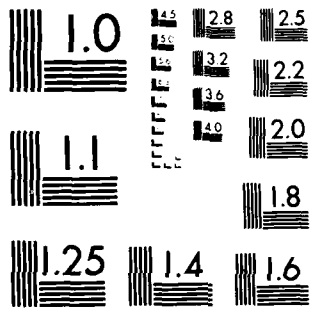
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STUDY OF AFRICAN TRYPANOSOMIASIS AND LEISHMANIASIS

FINAL REPORT

LTC M.J. Reardon, VC
I.E. Muriithi
LTC J.D. Chulay, MC
LTC L.D. Hendricks, MSC
B.T. Wellde
MAJ A. Wozniak, MSC
CPT R.F. Beach, MSC
CPT K.J. Linthicum, MSC

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Government of Kenya
USA Medical Research Unit - Kenya
Box 401
APO New York 09675

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| 20. ABSTRACT (Continue on reverse side if necessary and identify by block number) During the period 82 10 - 83 09 the investigators continued to monitor the anti- genic stability of parasites from western Kenya. Current evidence indicates that there was a significant antigenic shift in the 1980-81 outbreak. Epidemiology and treatment record analysis studies continued. A treatment center was opened in western Kenya situated north of the Lambwe Valley endemic area and to the east of the Ugandan epidemic area. This center will serve as a routine treatment facility and research facility for the evaluation of standard drugs available and USMRDC developed drugs effective in screens against human African trypanosomiasis. An experimental compound WR 163577 is being evaluated in the goat model against T. brucei infection. | | |

PROJECT 3M16270A871

WORK UNIT 162 VACCINE DEVELOPMENT IN TRYPANOSOMIASIS

1 OCTOBER 1982-30 SEPTEMBER 1983

INVESTIGATORS:

PRINCIPAL: LTC M.J. REARDON, VC

I.E. MURIITHI

ASSOCIATES: LTC J.D. CHULAY, MC

LTC L.D. HENDRICKS, MSC

B.T. WELLDE

MAJ A. WOZNIAK, MSC

CPT R.F. BEACH, MSC

CPT K.J. LINTHICUM, MSC

PROGRESS IN TRYPANOSOMIASIS RESEARCH

Epidemiology Survey

Data collected during the 1981-82 survey is being collated and processed. Medical records of sleeping sickness patients have been screened for treatment regimen, relapses, etc. Followup interviews and examinations have been performed on confirmed cases. Over seven hundred individuals have been identified as having sleeping sickness between 1961 and 1983. The deaths are being examined separately in an attempt to determine cause and relationship to sleeping sickness and/or treatment. Likewise various treatment regimens followed over the years are being examined for patterns of cure, relapse, drug reaction and fatalities. These data will be used to formulate protocols for treatment of patients at the Alupe treatment center.

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Alupe Treatment Center

A physician was assigned to head the center and began organizing the staff in late September. Equipping the facility and training the staff is progressing on schedule. It is anticipated that initial studies will center around the documentation of WHO approved treatment regimens in an effort to compile reliable data to support future protocols.

Lambwe Valley Trypanosome Studies

Studies conducted jointly with the Kenya Trypanosomiasis Research Institute have indicated that the parasites involved in the 1980-81 epidemic were different than those identified in the valley between 1973 and 1979. Isoenzyme analysis and serum neutralization studies are in agreement. Recent cases identified from outside the valley have been caused by parasites identified as being of the valley types of 1973-79 indicating that the dissemination occurred before the recent epidemic. These studies are continuing at the present time. To date there have been 46 confirmed cases in calendar 1983.

PROGRESS IN LEISHMANIA RESEARCH

Biochemical Characterization of Kenyan Leishmania Isolates

Cellulose acetate electrophoresis (CAE) is now being used to characterize Leishmania isolates. Unknowns are electrophoretically separated alongside already characterized control isolates then stained for specific enzymes to produce zymograms. The location of enzyme bands on such zymograms (migration distance from the origin) can be compared and the similarity of the unknown and one of the controls determined visually. At present, characterization is based on such comparisons using 5 different enzymes: PGI - phosphoglucoisomerase, G6PDH - glucose-6-phosphate dehydrogenase, ME - malic enzyme, PGM - phosphoglucomutase and ASAT - aspartate aminotransferase. Our controls include the following:

| NLB # | Taxonomic identity | origin/characterized at |
|-------|--------------------|---|
| 005 | <u>L. adleri</u> | Kenya/Liverpool School Trop Medicine |
| 070 | <u>L. major</u> | Jericho Valley/Hadassah Med School |
| 061 | <u>L. donovani</u> | Ethiopia/Hadassah Med. School |

Nine additional enzymes, for a total of 15, will be screened in an effort to identify which systems most clearly differentiate our control strains. It is hoped that as few as 4 well chosen enzymes will differentiate all *Leishmania* occurring in Kenya. This estimate is based on the observations of Dr. Richard Kreutzer, who has indicated that 4 enzymes are sufficient to separate all New World species of *Leishmania* he has examined - this includes much of the WRAIR *Leishmania* Bank.

A CAE project, already underway involves characterization of 6 isolates from 5 different species of rodents captured during a leishmaniasis reservoir study. Preliminary results are shown below.

Leishmaniasis Reservoir Study: Characterization of Isolates
from Small Rodents

| ISOLATE | RODENT | | | | |
|---------|--------------------|------|-------|------|------|
| NLB# | GENUS | PGI | G6PDH | PGM | ASAT |
| 095 | <u>Arvicanthus</u> | III* | IV | VIII | XI |
| 089 | <u>Tatera</u> | III | IV | VIII | XI |
| 098 | <u>Mastromys</u> | III | IV | VIII | XI |
| 088 | <u>Aethiomys</u> | III | IV | VIII | XI |
| 057 | <u>Taterillus</u> | III | IV | VIII | XI |
| ===== | | | | | |

CONTROLS

| | | | | | |
|-----|--------------------|-----|------|------|----|
| 005 | <u>L. adleri</u> | I | IV** | VI | IX |
| 061 | <u>L. donovani</u> | II | V | VIII | X |
| 070 | <u>L. major</u> | III | IV** | VIII | XI |

* for each enzyme a different Roman numeral is assigned to each different banding pattern.

** same band pattern for G6PDH

A similar analysis is also being applied to sandfly isolates. All projects remain in the pilot study category until more enzymes are screened and in some cases better replicates of those enzymes already examined are produced.

Band migration will eventually be quantified, all bands being assigned a relative migration value (R_f) which indicates migration distance as a proportion of the distance migrated by a reference band.

Localization of Leishmania Donovanii In Experimental Infected Sandflies: An Indicator of Vector Competence

Only three of the more than forty sandfly species occurring in Kenya are thought to transmit Leishmania donovani. These are Phlebotomus martini, Phlebotomus celiae and Phlebotomus vansomeranae. The vector competence of these species reflects their anthropophilic biting behavior and their ability to sustain L. donovani in the foregut of the alimentary system, from where the promastigote forms of the parasite are probably transmitted during bloodfeeding. The present study compared the fate of L. donovani in P. martini and Sergentomyia schwetzi, a nonvector sandfly. At regular intervals, following infection, both species were dissected to determine if and when promastigotes moved from the mid-gut to the head of the insect. Prior to dissection flies were also allowed to feed on hamsters thereby correlating parasite localization in the alimentary system with transmission of L. donovani. The results suggest that anterior migration is a prerequisite for transmission of L. donovani and that the physiological conditions which promote such transmission-favoring movement do not occur in the nonvector species.

Leishmania major in Kenya (East Africa): Transmission to a Human by Bite of a Naturally Infected Phlebotomus duboscqi Sandfly

We isolated Leishmania from a Phlebotomus duboscqi female captured in Baringo District, Kenya, and from papular lesions that developed at sites where this sandfly had fed on a human. When characterized by cellulose acetate electrophoresis (8 enzymes examined) these isolates proved to be identical to known Leishmania major strains from a human and a rodent (Arvicanthus sp.) and different from Leishmania donovani and Leishmania adleri which also occur in Baringo. This is the first case of human cutaneous leishmaniasis caused by L. major reported from Kenya.

Comparison of Three Culture Media for Isolating Leishmania donovani from Splenic Aspirates in Kenyan Visceral Leishmaniasis

Three culture media were compared for their sensitivity in isolating Leishmania donovani from splenic aspirates from patients with visceral leishmaniasis. A total of 151 splenic aspirates were obtained from 18 patients before, during and after chemotherapy. Aspirates were cultured in Schneider's Drosophila medium supplemented with 20% fetal bovine serum (SCH), a rabbit blood-agar diphasic medium (NNN) overlayed with normal saline (NS), and NNN overlayed with SCH. Giemsa stained aspirate smears were microscopically

examined. Of the 77 aspirates that were positive by any method, 88% were positive on smears, 57% were positive in NNN/SCH, 29% were positive in NNN/NS, and 25% were positive in SCH. Microscopy plus culture gave complementary results. We suggest that for optimal diagnosis and evaluation of response to treatment of visceral leishmaniasis in Kenya, splenic aspirates should be examined by microscopy and cultured in NNN/SCH.

High-Dose Sodium Stibogluconate Treatment of Cutaneous Leishmaniasis In Kenya

Cutaneous leishmaniasis caused by Leishmania aethiopica usually responds poorly to conventional doses of pentavalent antimonial drugs. We treated three patients with cutaneous leishmaniasis acquired in Kenya, presumed or documented to be caused by L. aethiopica with intravenous sodium stibogluconate, 18-20 mg Sb/kg body weight twice daily for 30 days. All patients had a good response to treatment, with disappearance of parasites from skin smears and cultures after 14 to 27 days, clinical healing of the lesions, and no recurrence during a 3 to 18 month follow-up. Side effects of treatment were minor. We conclude that this high dose sodium stibogluconate regimen is safe and effective for treating cutaneous leishmaniasis caused by L. aethiopica in Kenya.

PROGRESS IN RIFT VALLEY FEVER RESEARCH

Mosquito Species Succession In A Dambo In An East African Forest

The mosquito larval and pupal fauna of a dambo in a primary forest in Nairobi Area, Kenya was monitored during the short rainy season. The relative density of the immature stages of 6 species was recorded daily for a 3 month period. Aedes (Aedimorphus) cumminsii mediopunctatus (Theobald), Ae. (Neomelaniconion) lineatopennis (Ludlow), and Ae. (Mucidus) sudanensis (Theobald) were the first 3 species collected following flooding. Culex (Culex) quasiguiarti (Theobald), Anopheles (Anopheles) coustani (Laveran) and Cx. (Cux.) theileri (Theobald), were collected beginning 16, 17 and 33 days respectively following flooding. Each of the 3 Aedes spp. disappeared after one generation. All populations decreased to zero, after day 48.

Transovarial Maintenance of Rift Valley Fever Virus In Kenya By
Aedes Lineatopennis mosquitoes

Rift Valley fever virus was isolated from reared adult male and female Aedes lineatopennis collected as larvae and pupae on a ranch in Kenya during November and December 1982. This suggests transovarial transmission of the virus and supports an hypothesis that the virus is maintained during the interepizootic periods by transovarial transmission in Aedes lineatopennis.

Blood Feeding Activity Of Mosquitoes At A Flooded Grassland Dambo
In Kenya

The biting activity of mosquitoes encountered after flooding of a grassland dambo in Kenya was examined using human and calf bait. A total of 2,319 female mosquitoes, representing 9 species, were collected during a 96 hr period at human bait and a 48 hr period at calf bait. Aedes lineatopennis was the most commonly captured species. It represented 85% of the specimens collected at human bait and 96% of the specimens collected at calf bait. Diel biting activity was established for Ae. lineatopennis, Ae. cummingsii mediopunctatus and Ae. dentatus.

RECOMMENDATIONS

African Trypanosomiasis

It is recommended that human treatment studies and Lambwe Valley monitoring continue. The use of the goat model should be expanded and refined.

Leishmaniasis

Drug efficacy and pharmacokinetic studies should continue on currently available compounds until such time as new compounds or new formulations are available for field trials. Vector-reservoir field studies should be expanded. Controlled biochemical typing, morphologic taxonomy and transmission studies should be implemented as colony raised sandfly become available.

Rift Valley Fever

Efforts should continue along present lines of investigation since present data is preliminary.

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Manuscripts In Press

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Manuscripts Submitted

1. Beach, R.F., Kiilu, G., Hendricks, L.D. and Leeuwenburg, J.: Leishmania major in Kenya (East Africa): Transmission to a Human by Bite of a Naturally Infected Phlebotomus duboscqi Sandfly.

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